Claim 4/7/91

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:

BARCLAY

Serial No.: 07/580,778

Filed: September 11, 1990

Atty. File No.: 2391-1

For: "PROCESS FOR THE HETEROTROPHIC PRODUCTION OF MICROBIAL PRODUCTS WITH HIGH CONCENTRATIONS OF OMEGA-3 HIGHLY UNSATURATED FATTY ACIDS"

Group Art Unit: 188

Examiner: C. Geckle

DECLARATION OF WILLIAM R. BARCLAY (under 37 C.F.R. § 132)

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, William R. Barclay, declare as follows:

1. I graduated from St. Mary's College with a Bachelor of Science in Biology in 1971.

2. I graduated from the University of Wisconsin, Madison, with a Master of Science in Water Resource Management in 1974.

3. I graduated from the University of California, Davis, with a Doctor of Philosophy in Aquatic Ecology in 1981.

4. I was a Postgraduate Research Biologist with Scripps Institution of Oceanography at the University of California, San Diego, from 1981-1982 where I conducted research on the production of polysaccharides by soil algae and on controlling the bioflocculation potential of microalgae.

5. I was a Postdoctoral Fellow with Cooperative Institute for Research in Environmental Science at the University of Colorado, Boulder, from 1982-1983 where I conducted research on the production of polysaccharides, phenolics and organosulfur compounds by microalgae.

6. I was a Staff Scientist from 1983-1986 and a Senior Scientist from 1986-1987 with Solar Energy Research Institute,

Golden, Colorado, where I developed technology to produce liquid fuels from microalgae biomass.

7. I have been President and Director of Research for Phycotech, Inc. from 1987 to present, during which time I isolated and developed microbial strains for use in the production of omega-3 highly unsaturated fatty acids.

8. I am the inventor of the above-identified application and have reviewed the Office Action dated February 14, 1991.

9. With respect to the amendments to the specification of the above-identified application, I verify that all amendments of a numerical nature were to correct typographical errors and are not changes in the actual data. Specifically, the change on page 16 of "10%" to "15%" was to correct a typographical error. The change on page 54 of "39.0%" to "29.0%" was to correct a typographical error. The change on page 54 of "25.6%" to "15.6%" was to correct a typographical error.

10. With respect to the amendment to the drawings, I verify that the newly substituted Figure 6 for the originally filed Figure 6 is a correct representation of the data with respect to the strain identified by ATCC Accession Number 20889 which was misplotted in originally filed Figure 6. Specifically, in the originally filed Figure 6 the bars representing total fatty acids and omega-3 highly unsaturated fatty acids were misplotted for ATCC Accession No. 20889. These particular bars were misplotted, because a graphics plotting program was used to generate Figure 6 and erroneous data was entered into the program.

11. With respect to newly added Claims 54, 55, 58 and 59, I verify that the sodium concentration of the medium as claimed in these claims corresponds to the data points plotted in Figure 7. Specifically, these data points correspond to the raw data set forth in Table 1 attached hereto and incorporated herein by reference in its entirety. I further verify that the raw data of

Table 1 is the data generated in Example 8, the results of which are illustrated in Figure 7.

12. I certify that sea water has a sodium concentration of about 10.7 g/l.

13. With respect to newly added Claims 56 and 62, I verify that the claimed omega-3 highly unsaturated fatty acid content of greater than about 6.7 percent of total cell dry weight corresponds to the data generated in Example 7, the results of which are illustrated in Figure 6. Figure 6(a), attached hereto and incorporated herein by reference in its entirety, is a plot of the raw data from Example 7. When the raw data for the omega-3 highly unsaturated fatty acid content of the microorganism strains of the present invention (identified as ATCC Accession Numbers 20888 and 20889) and of the prior art strains (identified by ATCC Accession Numbers 28211, 34304, 28210, 24473 and 28209) are plotted, a comparison among the strains of the omega-3 highly unsaturated fatty acid content is difficult, because some of the resulting bars in the graph are so small (See Figure 6(a)). Therefore, the data was plotted as relative data in Figure 6, normalized to the corresponding values for ATCC Accession Number 28211. The data are more readily comparable when plotted in such a manner. From Figure 6, it is readily apparent that the strains of the present invention (ATCC Accession Numbers 20888 and 20899) have an omega-3 highly unsaturated fatty acid content which is greater than the prior art strains by a magnitude of two to three times. I further verify that the raw data set forth in Table 2 is accurate and is the data generated from Example 7. The omega-3 highly unsaturated fatty acid content (expressed as percent of total cell dry weight) can be calculated by dividing the omega-3 highly unsaturated fatty acid concentration, expressed as mg/l, (Column 6) by the total cell dry weight concentration, expressed as mg/l (Column 2).

14. Long teaches and claims use of extracted fatty acids from the strains taught in the Long PCT application. The strains Long discloses to produce omega-3 highly unsaturated fatty acids have a very low omega-3 highly unsaturated fatty acid content, and as such, the lipids must be extracted from the microbial biomass to provide a source which is concentrated enough to be biologically, physiologically and economically effective and useful. The omega-3 content of the strains Long teaches (published by others prior to Long's priority date of July 20, 1987) are as follows:

Strain	Omega-3 Highly Unsaturated Fatty Acids As % Dry Weight	Reference
Nitzschia	0.22 - 0.96%	Tornabene et al. (1974)
Crypthecodinium	1.5%	Harrington & Holtz (1968)
Pythium	0.75 - 0.84%	Haskins et al. (1964)
Yeast	0.0%	No yeast produce omega-3 Highly Unsaturated Fatty Acids
Thraustochytrids	?	No published lipid content information

(The references cited above are disclosed in a Supplemental Information Disclosure Statement submitted herewith.)

Some of these strains are mentioned in the above-identified application as prior art strains. Applicant's present application teaches extensively why one would not want to use these prior art strains. Specifically, the prior art strains have a very low omega-3 fatty acid content (as percent of total cell dry weight), therefore, it is necessary to extract the fatty acids in order to have a product which would be biologically effective in the products listed. Furthermore, extractions of the fatty acids is an expensive process and thus is not commercially preferred, because

the expense is passed on to the end omega-3 fatty acid product. Long does not teach use of whole cells nor methods of cultivation to produce high omega-3 highly unsaturated fatty acid contents in whole cells. As a point of comparison, one of the strains taught in the above-identified application produces, at a minimum, 6.7 percent of its total cell dry weight as omega-3 highly unsaturated fatty acids. Therefore, only 6 grams of whole cells per day need to be fed to a laying hen to produce an omega-3 highly unsaturated fatty acid enriched egg according to the method outlined in Example 14. Using the highest omega-3 highly unsaturated fatty acid contents for the strains taught by Long, it would be necessary to feed about 42, 26 or 48 grams of dry cells per day of Nitzschia, Crypthecodinium, or Pythium, respectively. Since laying hens only eat 100 grams per hen per day, this would require a laying hen to eat an equivalent of about 26 percent to about 48 percent of its diet of the microorganism strains taught by Long in order to achieve the same effect. Replacing this amount of the hen's diet with whole cells of the strains taught by Long presents several problems: 1) it would most likely lead to nutritional imbalance in the hens and cause a cessation or serious reduction of egg production, because so much of the laying hen's normal diet will be substituted by the microbial cells; and 2) it would cost four to eight times as much to achieve the same effect using the strains taught by Long as achieved by feeding a laying hen the microorganism strains taught and claimed by the present invention.

claims not so limited in all cases

no evidence

15. I hereby declare that all statements made herein of my own are true and that all statements made on information or belief are believed to be true; and further that the statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application of any patent issuing thereon.

Date: 5/20/91 By: William R. Barclay
William R. Barclay

wp-cmk/2391-1.DEC/D1/BLS

TABLE 1

Omega-3 highly unsaturated fatty acid contents (% dry weight) for the strains illustrated in Figure 7.

Sodium Conc (g/l)	Omega-3 HUFA Content (% dry weight) ATCC 20888*	ATCC 20891*	ATCC 208210**	ATCC 28209**
0.83	9.8			
0.97	7.2			
1.37	9.2			
1.66	7.8			
2.15	8.1	4.3	3.4	
2.65	6.9	5.3	3.8	0.7
4.61	5.6	5.9	6.0	0.8
6.58	4.4	6.4	5.7	1.2
8.54	3.3	5.5	5.7	1.1
10.51	5.7	5.5	2.5	0.6

* strains isolated by the selection method of this invention
** prior art strains

TABLE 2

Raw data from Example 7 from which Figure 6 was derived. For Figure 6, the data were normalized by setting the data of strain ATCC 28211 equal to 1. The omega-3 highly unsaturated fatty acid content (as percent dry weight) can be calculated by dividing the omega-3 highly unsaturated fatty acid concentration (mg/l) by the dry weight concentration (mg/l).

ATCC Strain	Dry Wt. mg/l	Relative Dry Wt.	Fatty Acids mg/l	Relative Fatty Acid	n-3 HUFA mg/l	Relative n-3 HUFA	n-3 HUFA Content As Percent Dry Weight
20888[a]	4100	2.1	841	5.3	318	3.0	7.8
20889[a]	4680	2.4	720	4.4	313	3.0	6.7
28211[b]	1960	1.0	162	1.0	105	1.0	5.4
34304[b]	2070	1.1	142	0.9	91	0.9	4.4
28210[b]	1720	0.9	129	0.8	84	0.8	4.9
24473[b]	1180	0.7	87	0.5	37	0.4	3.1
28209[b]	1040	0.5	54	0.3	21	0.2	2.0

[a] = strains isolated by the selection method of the present invention

[b] = prior art strains

FIGURE 6(a).

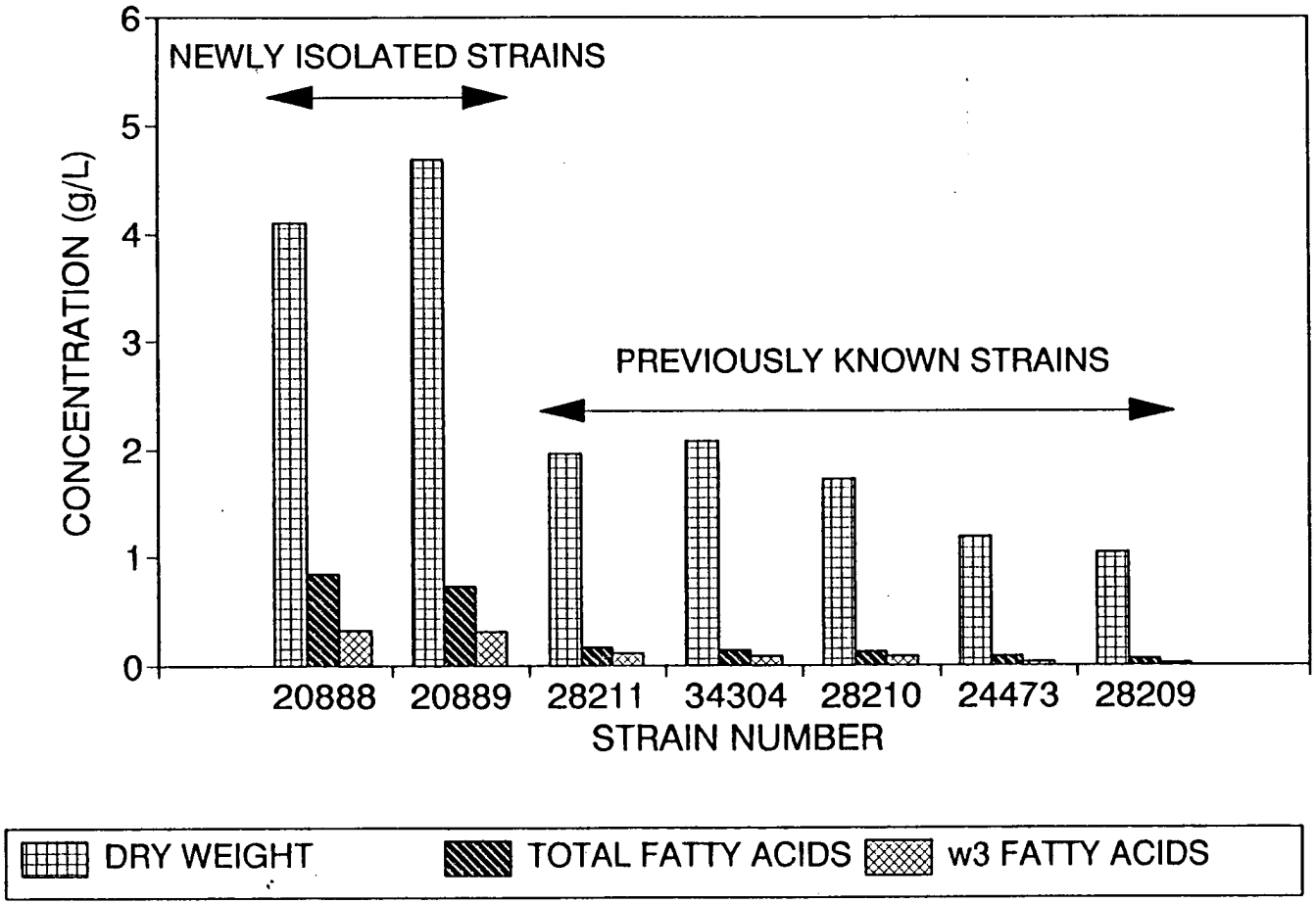
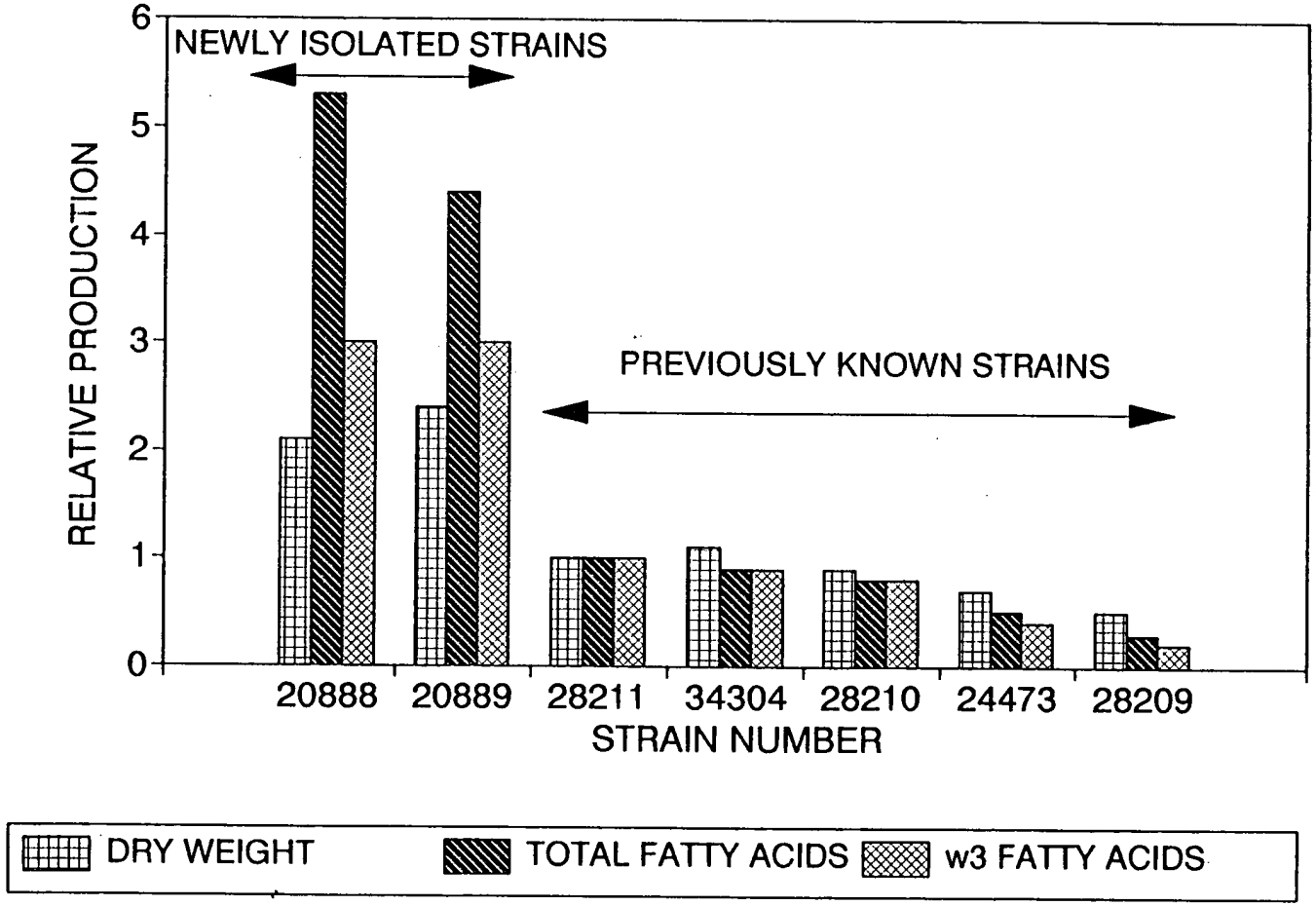

NEWLY ISOLATED STRAINS
PREVIOUSLY KNOWN STRAINS
CONCENTRATION (g/L)
0
1
2
3
4
5
6
20888
20889
28211
34304
28210
24473
28209
STRAIN NUMBER
DRY WEIGHT
TOTAL FATTY ACIDS
w3 FATTY ACIDS

FIGURE 6.


NEWLY ISOLATED STRAINS
PREVIOUSLY KNOWN STRAINS
RELATIVE PRODUCTION
0
1
2
3
4
5
6
20888
20889
28211
34304
28210
24473
28209
STRAIN NUMBER
DRY WEIGHT
TOTAL FATTY ACIDS
w3 FATTY ACIDS